GENETIC ANALYSIS OF RECOMBINANT INBRED LINES FOR TOTAL GRAIN PROTEIN CONTENT AND GRAIN YEILD IN RICE (*Oryza sativa* L)

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ABSTRACT

Grain protein content in rice is an important nutritional parameter and development of quality rice with enhanced nutrition is very essential for eradication of malnutrition in the developing world. Evaluation of genetic variability is the source for any crop improvement programme, the success of which depends on sufficient genetic variability among genotypes permitting in effective selection. Genetic variability for many of the quantitative traits is enormously available in rice and variability can also be created artificially by means of several breeding techniques. Rice recombinant inbred lines were developed for total grain protein content and other yield related traits. Recombinant inbred lines, being the derivatives of two genetically diverse parents, serve as good source of genetic diversity as well as permanent mapping population to study this complex quantitative trait controlling protein content in rice. Several transgressive segregants superior to the parents for total grain content and other yield related traits have been identified.

KEYWORDS: Variability, Total Grain Protein Content (TPG), Recombinant Inbred Line (RIL)

INTRODUCTION

Rice is the most important cereal crop on the earth and is used as staple food by more than half of the world's population. It is estimated that about 23 per cent of global per capita energy and 16 per cent of protein are supplied by rice crop alone (FAOSTAT, 2001). Total grain protein (TGP) in seeds is the important nutrient analyzed in rice and its content ranges from 4.5 to 19.3 percent in *Oryza sativa* species and 10.2–15.9 percent in *Oryza glaberrima* species (Juliano and Villareal, 1993). TGP of rice grain influences the taste of cooked rice and also affects the physical and chemical properties of cooked rice (Wan et al. 2004). Earlier genetic studies have shown that TGP is quantitatively inherited (Shenoy et al. 1991; Shi et al. 1999) and is sensitive to environmental conditions like the level of nitrogen fertilization (Perez et al. 1996). This in turn has contributed to the slow progress of breeding efforts to improve TGP (Aluko et al. 2004).

Habitually, plant breeding in rice was more focused towards the development of high yielding and stress tolerant varieties. Increased demand for quality rice with enhanced nutrition has driven breeding objectives to some extent towards rice quality improvement. Most present day cultivated rice varieties are having low protein content of 7 to 8 percent (FAO, 2004) and hence higher grain yield with better taste and high nutrition are the main aspects of rice quality improvement programs. But, in contrast these characters are controlled by quantitative trait loci (QTLs) showing continuous phenotypic variation in the progeny (Yano and Sasaki, 1997).

Recombinant inbred lines, being the derivatives of two genetically diverse parents, serve as good source of genetic diversity as well as permanent mapping population to study this complex quantitative trait controlling protein

content in rice. Recombinant inbred lines (RILs), the product of hybridization and repeated selfing serve as a good source of genetic variability, resulting in many superior recombinants as well as to break the undesirable linkages along with protein content, high yielding recombinants and many a times, producing transgressive segregants superior than parents.

In this regard the present research was initiated to enhance the TGP and to identify DNA markers associated with protein content. Since there is tight linkage between yield and TGP reported, a large number of RILs populations were developed to break the linkage. The objective was to identify recombinants with high yield and high protein in addition to good grain quality parameters. Evaluation of genetic variability forms the basis for any crop improvement programme, the success of which depends on sufficient genetic variability among genotypes so as to permit effective selection. Apart from available genetic variability in nature, variability is created artificially by means of hybridization and mutation. TGP is an important component determining quality in cereals, where increased TGP in grains leads to enhanced nutrition in the consumers.

MATERIALS AND METHODS

Plant Materials

Rice recombinant inbred population (RIL) consisting of 1800 F_8 individuals were developed at Marker Assisted Selection laboratory, UAS, Bangalore, by single seed descent method from a cross between BPT-5204 and HP-14. RILS along with parents were first sown in nursery and twenty five days old seedlings were transplanted in the main experimental plots. Inbred lines were planted with 25 cm spacing between the rows and 20 cm between lines in Augumented design (Federer *et al.*, 1961) at Regional Research Station, Zonal Agricultural Research Station Mandya, and University of Agricultural Sciences. The crop was raised in irrigated condition during *kharif* 2009 and summer 2010 with regular cultural practices to ensure uniform crop stand as per the package of practices.

Observations and Data Analysis

Observations were recorded on five randomly selected plants per each RIL for the quantitative traits, days to 50% flowering, plant height, panicle length, Number of tillers, Number of productive tillers, panicle weight, Grain yield/plant, Days to maturity, grain length and grain breadth. Total grain protein content was estimated using near infrared reflectance spectroscopy (Bruker, NIR system, Germany) system at Marker Assisted Selection Laboratory, Dept. of genetics and Plant Breeding, University of Agricultural sciences, Bangalore. The analysis of variance (ANOVA) (Federer 1961) was carried out to separate variability components from total variability as per augmented design using Statistical Package AUGDES1 developed by Tamil Nadu Agricultural University, Tamil Nadu. Basic statistics were applied using statistical software SPAR 2 developed by Indian Agricultural Research Institute. Distribution of quantitative traits for the entire population was performed using *STATISTICA* software package.

RESULTS AND DISCUSSIONS

Analysis of variance for all the studied quantitative characters showed significant differences for the entire RIL's population studied. Days to flowering, grain length and grain breadth traits were found to be significant (Table 2). Results showed low, moderate to high coefficients of variability for the traits studied under field conditions. Based on the mean performance of traits namely, total grain protein, days to flowering, grain yield/plant six RILs, HPR 0292, HPR 0922, HPR 1139, HPR 1623, HPR 1749 and HPR 1805 were selected (Table 3). With respect to plant height and protein %, three RILs viz., HPR 0288, HPR 1198 and HPR 1230 have been selected (Table 4). The frequency distributions of analyzed

traits are presented in figure 1. Results of RILs showed normal distribution of for total grain protein, plant height, total tillers, productive tillers, grain yield/plant and days to maturity. About 550 RILS recorded the total grain protein content of 10-12% and more than 12% of total grain protein content was observed among 153 RILs. With respect to days to flowering about 700 RILs were in the group of 90 to 95 days and about 230 RILs were in the group 80-85 days to flowering.

Analysis of genetic parameters of entire RIL population showed wide range of variability for the characters studied (Table 1). Variability for total grain protein was observed in the range of 1.530 to 22.42 with an average of 9.081, Days to 50% flowering was ranged from 80 to 120 days with a mean of 93.78, Plant height ranged from 44 to 132 cm with a mean of 93.10, Panicle length ranged from 13 to 27 cm with a mean of 21.02, Total tillers ranged from 2 to 21 with a mean of 8.78 and number of productive tillers from 1 to 18 with a mean of 7.40, Panicle weight ranged from 0.70 to 6.20 gm with a mean of 3.13, Grain yield ranged from 4 to 35 gm with a mean of 21.59, Days to maturity ranged from 110 to 152 days with a mean of 125.15, Grain length ranged from 6.4 to 10.1 cm with a mean of 7.75 and grain breadth ranged from 1.9 to 5.10 cm with a mean of 2.39.

Correlation Analysis

Variability studies indicate the potential contributors for grain yield, but fail to enlighten on the nature and extent of relationship between grain yield and its contributing characters, as well as among themselves. Information to this effect could be obtained through correlation co-efficient, which throws light on traits to be considered for augmenting the grain yield. Genetic correlation between various plant characters arises because of linkage, pleiotropy or developmental interrelatedness (Lebreton *et al.*, 1995) including functional relationship. Since most of the characters associated with nutrition enhancement in rice are quantitative in nature, understanding the association between these traits is essential for simultaneous selection of traits. Information on the nature and magnitude of association among various traits facilitate the breeders in identification and selection of superior genotypes for enhanced nutrition. Correlation coefficients were computed to find the anticipation amongst characters using the formula given by Webber and Moorthy (1952) and were then compared with 'r' values at n-2 degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance. The phenotypic correlation coefficients among the characters studied are presented for correlation of TGP content and grain yield with other important traits separately. There was no positive significant correlation between TGP and other quantitative traits but grain yield had a positive significant correlation with days to maturity (0.1245) and negative association with awn type (-0.07) and plant colour (-0.1801) in the studied recombinant inbred population.

CONCLUSIONS

The availability of genetic variability is a prerequisite to initiate breeding programme in any crop for facilitating selection. Breeding for nutritional enhancement is rather highly difficult compared to other quantitative traits. Hence hybridization followed by selection in the recombinant lines is very important in the development of high yielding varieties with enhanced nutrient contents.

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APPENDICES

**Table 1: Range Mean and Estimates of Genetic Characters in the
Recombinant Inbred Population under Submerged Conditions**

Characters	Range		Mean	SE m	PCV	GCV	H²
	Min	Max					
Total grain protein (%)	1.53	18.42	9.11	0.05	24.16	24.09	99.42
Days to 50% flowering	80.00	120.00	93.77	0.12	5.79	5.56	92.18
Plant height (cm)	44.00	132.00	93.09	0.36	16.83	16.72	98.69
Panicle length (cm)	13.00	27.00	21.02	0.05	10.96	10.88	98.57
Number of tillers	2.00	21.00	8.78	0.06	32.15	31.11	93.66
Number of productive tillers	1.00	18.00	7.40	0.06	37.30	36.40	95.35
Panicle weight (gm)	0.70	6.20	3.13	0.02	28.07	28.07	100.1
Yield per plant (gm)	4.00	35.00	21.60	0.17	33.59	33.51	99.57
Days to maturity	110.00	152.00	125.14	0.13	4.44	4.27	92.62
Grain length (cm)	6.40	10.10	7.76	0.01	7.35	7.37	100.8
Grain Breadth (cm)	1.90	3.00	2.41	0.02	10.07	9.99	98.37

SE m-Standard error, SD=Standard deviation, CV=Coefficient of variation (%),
Phenotypic Coefficient of variation (%), Genotypic Coefficient of variation (%),
Broad sense Heritability (%)

Table 2: Analysis of Variance for Quantitative Characters [TGP=Total Grain Protein (%),
DFF= Days to 50% Flowering, GM= General Mean, PHT= Plant Height (cm),
PL=Panicle Length (cm), NT=Number of Tillers, PT=Number of Productive Tillers,
PW=Panicle Weight (gm), YPP=Yield per Plant (gm), DM=Days to Maturity, GL=Grain Length (cm),
GB=Grain Breadth (cm)] of RIL's under Submerged Conditions

Source of Variation	df	Mean sum of squares										
		TGP %	DFF	PHT	PL	NT	PT	PW	YPP	DM	GL	GB
Entries	1829	0.9388	131.0897**	0.3621	0.5985	0.2887	0.5364	1.0411	0.7084	0.0378	4.9415**	9.1248**
Checks	1	0.1394	10336.61	7.3	0.0906	8.1569	13.0358	1.0746	10.2888	0.0318	1.6974	32.3461
Lines	1827	0.9335	119.7424	0.35	0.5938	0.2748	0.5168	1.0412	0.6991	0.0083	4.9411	9.1149
Checks v/s Lines	1	11.4265	10657.1	3.43	9.6517	17.7702	23.8492	0.735	8.0221	53.9306	8.8275	4.0446
SE m b/w test entries		2.3954	0.4773	26.06	2.9721	5.2456	3.779	0.8628	8.6442	58.9284	0.2574	0.228
SE m b/w checks		0.7575	0.1509	8.24	0.9399	1.6588	1.1947	0.2728	2.7335	18.6348	0.0814	0.0721
CD at 5% b/w test entries		7.11	1.4174	77.41	8.8267	15.5787	11.2197	2.5624	25.672	175.0085	0.7646	0.6772
CD b/w checks		2.24	0.4482	24.48	2.7912	4.9264	3.548	0.8103	8.1182	55.3426	0.2418	0.2141
CD b/w Test v/s check.		5.27	1.0512	57.41	6.546	11.5535	8.3207	1.9003	19.0388	129.7898	0.567	0.5022

Table 3: List of Selected Fine Grain RIL's of BPT 5204 × HP 14 Based on Protein %,
Days to Flowering and Grain Yield/Plant under Wetland Conditions {TGP: Total Grain Protein (%),
DFF: Days to Flowering, PL: Panicle Length (cm), NT: Number of Tillers, PT: Productive Tillers,
PW: Panicle Weight (gm), YPP: Grain Yield/Plant (gm), DM: Days to Maturity, GL: Grain Length (cm),
Grain Breadth (cm), GT: Grain Type}

HPR RIL	TGP (%)	DFF	PHT (cm)	PL (cm)	NT	PT	PW (gm)	YPP (gm)	DM	GL (cm)	GB (cm)	Grain Type
HPR 0292	11.36	92.00	103.00	18.00	9.00	4.00	3.20	22.00	122.00	9.60	2.20	Fine
HPR 0922	12.18	91.00	95.00	18.00	18.00	16.00	1.70	25.00	123.00	7.50	2.20	Fine
HPR 1139	12.02	90.00	107.00	24.00	9.00	9.00	3.20	26.00	122.00	9.70	2.20	Fine
HPR 1623	11.30	95.00	102.00	23.00	10.00	6.00	3.20	23.00	127.00	7.10	2.20	Fine
HPR 1749	13.20	95.00	91.00	18.00	7.00	7.00	2.70	34.00	127.00	7.10	2.10	Fine
HPR 1805	11.91	92.00	113.00	20.00	8.00	6.00	4.20	22.00	124.00	7.60	2.20	Fine

Table 4: List of Selected fine Grain RIL's of BPT 5204 × HP 14 Based on Protein % and
Plant Height under Wetland Condition TGP: Total Grain Protein (%),
DFF: Days to Flowering, PL: Panicle Length (cm), NT: Number of Tillers, PT: Productive Tillers,
PW: Panicle Weight (gm), YPP: Grain Yield/Plant (gm), DM: Days to Maturity,
GL: Grain Length (cm), Grain Breadth (cm), GT: Grain Type

HPR RIL	TGP (%)	DFF	PHT (cm)	PL (cm)	NT	PT	PW (gm)	YPP (gm)	DM	GL (cm)	GB (cm)	Grain Type
HPR 0288	11.80	97.00	82.00	21.00	4.00	4.00	4.10	10.00	127.00	8.10	2.10	Fine
HPR 1198	11.18	97.00	75.00	21.00	14.00	10.00	2.70	14.00	129.00	7.90	2.20	Fine
HPR 1230	11.65	92.00	87.00	18.00	6.00	6.00	2.70	16.00	124.00	7.00	2.50	Fine

Table 5: Correlation Coefficient among Traits (TGP=Total Grain Protein (%), DFF= Days to 50% Flowering, PHT= Plant Height (cm), PL=Panicle Length (cm), NT=Number of Tillers, PT=Number of Productive Tillers, PW=Panicle Weight (gm), YPP=Yield per Plant (gm), DM=Days to Maturity, GL=Grain Length (cm), GB=Grain Breadth (cm), AW=Awn type, CL= Plant colour) Studied under Wetland Conditions in RILs of BPT-5204/HP-14

	TGP%	DFF	PHT	PL	NT	PT	PW	YPP	DM	GL (cm)	GB (cm)	AW	CL
TGP%	1.0000												
DFF	-0.0532	1.0000											
PHT	0.0375	-0.0250	1.0000										
PL	0.0023	0.0306	0.4572	1.0000									
NT	-0.0366	-0.0935**	-0.0031	0.0479	1.0000								
PT	0.0235	-0.1319**	0.0209	0.0224	0.5349**	1.0000							
PW	-0.0295	0.1020**	0.2499	0.1946**	-0.0044	-0.0251	1.0000						
YPP	-0.0209	0.1123**	0.2485	0.2077**	0.2791**	0.2348**	0.3406**	1.0000					
DM	-0.0262	0.9787**	-0.0272	0.0196	-0.0937**	-0.0823**	0.0886**	0.1245**	1.0000				
GL (cm)	0.0197	-0.1027**	0.0153	0.1377**	0.0825**	0.0958**	0.0251	0.0457	-0.1045**	1.0000			
GB (cm)	-0.0069	0.0337	-0.0143	0.0125	-0.0633*	-0.0653*	0.0775*	0.0526	0.0273	-0.0004	1.0000		
AW	0.0328	-0.0694*	0.0943**	0.0187	-0.0660*	-0.0518	-0.0131	-0.0707**	-0.0677*	-0.1023**	-0.0140	1.0000	
CL	0.0310	-0.0433	0.0645*	0.0262	-0.2321**	-0.1352**	-0.0176	-0.1500**	-0.0537	-0.1361**	-0.0171	0.5035**	1.0000

* = Significant at 5%

** = Significant at 1%

Figure 1: Frequency Distribution of Quantitative Traits TGP=Total Grain Protein (%), DFF= Days to 50% Flowering, PHT= Plant Height (cm), PL=Panicle Length (cm), TT=Number of Tillers, PT=Number of Productive Tillers, PW=Panicle Weight (gm), GY=Yield per Plant (gm), DM=Days to Maturity, GL=Grain Length (cm), GB=Grain Breadth (cm), TW=Test Weight (gm) of High Protein Rice Recombinant Inbred Lines under Wetland Conditions